CHAPTER 18

ROSA® Aflatoxin P/N TEST KIT

18.1 GENERAL INFORMATION

The ROSA® P/N test kit uses lateral flow test strip technology that provides qualitative (equal to or less than a specified threshold) results.

18.2 PREPARATION OF EXTRACTION SOLUTION

The extraction solvent used in the ROSA® P/N test method is either a methanol/water (distilled or deionized) mixture consisting of 70 percent methanol (Reagent grade or better) and 30 percent water or 50 percent ethanol (Reagent grade or better) and 50 percent water.

a. Methanol/Water

(1) Using a graduated cylinder, measure 700 ml of methanol and place it into a clean carboy with spigot.

(2) Add 300 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (70 percent methanol and 30 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 7 parts methanol to 3 parts of deionized or distilled water.

b. Ethanol/Water

(1) Using a graduated cylinder, measure 500 ml of ethanol and place it into a clean carboy with spigot.

(2) Add 500 ml deionized or distilled water to the ethanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (50 percent ethanol and 50 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 1 part ethanol to 1 part deionized or distilled water.

18.3 PREPARATION OF TESTING MATERIALS

NOTE: A Negative and Positive Control must be run daily to verify performance of equipment and test strips.

a. Negative Control.

Add 100 µl of 70% methanol or 50% ethanol solution to 1.0 ml of AFQ Buffer to prepare Negative Control Diluted Extract.

b. Positive Control.

Prepare the Aflatoxin B1 Control by adding 3.0 ml of deionized or distilled water and 300µl of 70% methanol or 50% ethanol. Mix thoroughly.

NOTE: Store at 32-45 °F for up to one week, or freeze at -4 °F for 2 months.

c. Equipment Preparation.

(1) Incubator must be at 45±1°C (temperature indicator is green).

(2) Incubator must be clean and level.

d. AFQ Dilution Buffer.

Predispense 1.0 ml of AFQ Dilution Buffer into a micro-centrifuge tube for each sample to be tested.

e. Test Strips.

(1) Remove ROSA® P/N moisture resistant container from the refrigerator and allow it to reach room temperature to limit condensation.

(2) Remove only the number of strips to be used and return container to 32-45 °F storage. Strips are stable at room temperature for at least 12 hours.

NOTE: If blue desiccant packets turn white or pink, performance test the strips with Negative and Positive Controls before continued use.

18.4 EXTRACTION PROCEDURES

a. Transfer 50grams of ground sample into a clean extraction container.

b. Add 100 ml of the (70/30) methanol/water or (50/50) ethanol extraction solvent.

c. Shake for at least 30 seconds. Allow sample to settle 1 minute to obtain clarified sample extract.

NOTE: If particles are present after settling, filter or centrifuge to clarify sample extract. **To Filter:** funnel the extract through Whatman 2V (or equivalent) filter paper into a labeled collection container. **To Centrifuge:** transfer 1.0-1.5 ml of sample extract to a labeled micro-centrifuge tube and centrifuge for 10 seconds. Clarified extract is now ready for testing.

18.5 TEST PROCEDURES

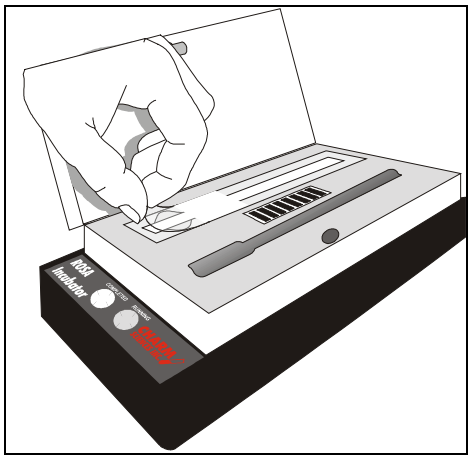
a. Sample Preparation.

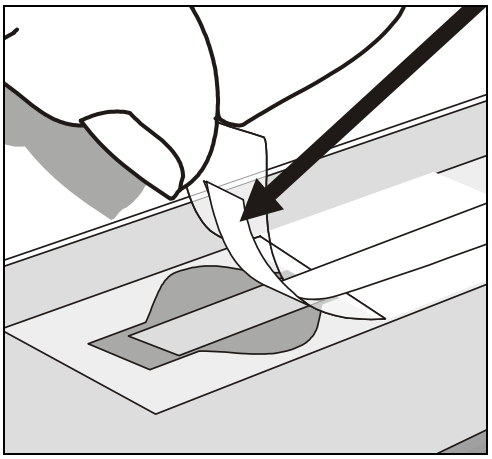
(1) Pipet 100 µl of clarified extract to a predispensed (1.0 ml AFQ Dilution Buffer), labeled micro-centrifuge tube, cap, and mix. This is the diluted extract.

(2) Label the test strip to identify sample.

(3) Open the incubator lid and place test strip in the ROSA-M Incubator with the flat side facing upward.

(4) While holding the strip flat on the incubator, use tab to peel tape back to the indicated line exposing the sample pad. Avoid bending back the white wick and sponge under the tape.

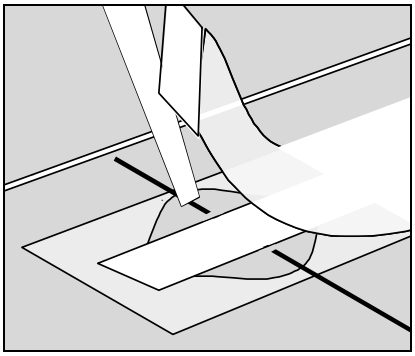




b. Sample Analysis.

(1) Pipet 300 µl of diluted extract into the side of the side of the strip sample compartment at the position indicated by the black line on the incubator.

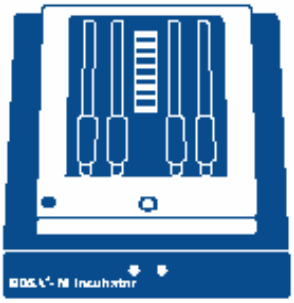
NOTE: Pipet very slowly.



(2) Reseal the tape over the sample pad compartment. When testing multiple samples, complete the peel, pipet, and reseal steps on each strip before going to the next strip.

NOTE: Add diluted extract to all strips within 1 minute. If a quad incubator is used, 4 samples can be incubated simultaneously.

(3) Close lid on the incubator and tighten the latch. The solid red timer light will automatically start when the lid is closed.



LF-INC4-45D: Quad incubator, 3-minute timer with display, set for 45° C for Test Strips

(4) Incubate for 3 minutes. After the incubation step is complete, a beeper will sound and the yellow "test complete" light will begin to flash.

(5) Remove strips and interpret the results. **Strips must be removed from the incubator and read within 2 minutes of incubation completion.** After strip removal, lower but do not latch the incubator lid.

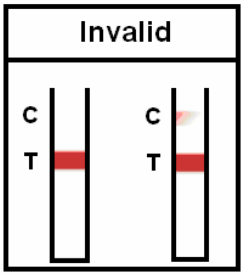
c. Visually Interpreting the Lateral Flow Test Strip.

Development of a Control Line indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded. A second preparation of the extract (using a fresh dilution) should be made and tested using another strip.

Note: The examples shown below depicting invalid, negative, and positive results is for illustration purposes only. Do not use these color bars as actual intensity measurement for determining if the sample is positive or negative.

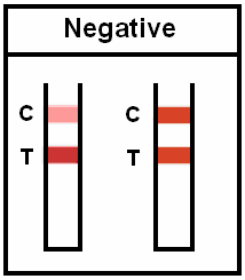
(1) Invalid Result.

A test is invalid if a Control Line is missing, smeared, or uneven, or if the Test Line is uneven. It is invalid if the diluted extract is obscuring either the Control (C) or Test Line (T).



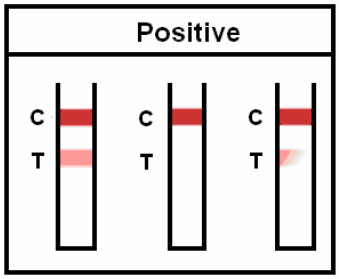
(2) Negative Result.

A sample containing aflatoxin residues less than or equal to 20 ppb will develop a Test Line that is darker or equal in intensity to the Control Line in the test area.



(3) Positive Result.

A sample containing aflatoxin residues in excess of 20 ppb will develop a Test Line that is lighter in intensity than the Control Line.



d. Interpreting the Lateral Flow Test Strip using the ROSA-M Reader.

(1) Insert a clean valid test strip into the ROSA-M Reader. Slide the strip into the slot, with the sample compartment in the up position, until it stops.

LF-ROSA READER-M: ROSA-M Reader supplied with calibrators.

(2) Read result on **AFLA** Channel (2-Line Mode) of the ROSA-M Reader. If desired, enter **Sample** and/or **Operator.** Press **ENTER** to read.

(3) **Result:** The ROSA-M Reader interprets the strip and displays either **NEGATIVE** or **POSITIVE.**

18.6 REPORTING AND CERTIFYING TEST RESULTS

a. Report results on the pan ticket and inspection log as being equal to or less than 20 ppb (≤ 20 ppb), or as exceeding 20 ppb (> 20 ppb), as applicable.

b. Certify results as being equal to or less than 20 ppb or exceeding 20 ppb, as applicable.

c. Refer to the Certification section of the handbook for more detailed certification procedures.

18.7 CLEANING LABWARE

a. Negative Tests (≤ 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used extraction mixing jars, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used extraction mixing jars and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used test strips and pipettes, for at least 5 minutes.

Pour off the liquid down the drain and place the materials in a garbage bag and discard.

18.8 WASTE DISPOSAL

a. Negative Results (≤ 20 ppb).

If the test result is negative (equal to or less than 20 ppb), dispose of any remaining liquid filtrate in the chemical waste container. Discard the sample slurry (ground material) into a plastic garbage bag for disposal.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the slurry (ground portion) remaining in the sample extraction jar must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, pour approximately 50 ml of bleach solution into the sample extraction jar and shake to mix with the sample slurry. After the slurry and bleach solution separate, handle the bleach rinse filtrate as a non-hazardous solution and dispose of by pouring the liquid down the down. Discard the sample slurry (ground portion) paper into a plastic garbage bag for disposal.

18.9 EQUIPMENT AND SUPPLIES

a. Materials Supplied in Test Kits.

Kits can be purchased that contain 20, 100, or 500 strips and include Control and AFQ Dilution Buffer.

(1) LF-APN-20 –

(a) 1 package containing 20 ROSA® strips packed in a moisture-resistant container.

(b) 1 Aflatoxin B1 20 ppb Control.

(c) 1 AFQ Dilution Buffer

(2) LF-APN-100 –

(a) 1 package containing 100 ROSA® strips packed in a moisture-resistant container.

(b) 1 Aflatoxin B1 20 ppb Control.

(c) 1 AFQ Dilution Buffer.

(3) LF-APN-500 –

(a) 5 packages containing 100 ROSA® strips packed in a moisture-resistant container.

(b) 5 Aflatoxin B1 20 ppb Controls.

(c) 5 AFQ Dilution Buffers.

b. Materials Required but not Provided:

(1) Sample grinder.

(2) Balance.

(3) Methanol - Reagent grade or better.

(4) Deionized or Distilled water.

(5) Sample extraction containers.

(6) 1.0 ml pipettor and pipette tips.

(7) 300 µl pipettor and pipette tips.

(8) 100 µl pipettor and pipette tips.

(9) 25 ml graduated cylinder.

(10) 1.5 ml micro-centrifuge tubes.

c. Optional Equipment and Supplies:

(1) Mini-centrifuge.

(2) Whatman 2V filter paper or equivalent.

(3) Filter funnel.

18.10 STORAGE CONDITIONS

a. Storage Conditions.

Test kits should be refrigerated between 32°- 45°F.

b. Precautions.

(1) Do not use the test kits beyond the noted expiration date.

(2) Prolonged exposure to high temperatures may adversely affect the test results.

(3) Do not open the desiccated canister until ready to use the strips.